REMARKS

Claims 1, 2 and 4-12 are now in the application. Claim 3 has been canceled without prejudice or disclaimer. Claims 1 and 2 have been amended to recite "a member selected from the group consisting of (a) adenosine 5'-triphosphate, (b) adenosine 5'-monophosphate and (c) a mixture of adenosine and inorganic phosphate" in place of "at least one member selected from the group consisting of adenosine 5'-triphosphate and adenosine 5'-monophosphate."

Claims 4-6 have been amended to recite "adenosine 5'-triphosphate or adenosine 5'-monophosphate or a mixture of adenosine and inorganic phosphate" to render these claims consistent with claims 1 and 2. Support for the recitation "mixture of adenosine and inorganic phosphate" can be found at page 8, lines 14-25. Claims 4-11 have been amended to recite "claims 1-2" in place of "Claims 1-3" in view of the cancellation of Claim 3. Basis for Claim 12 can be found for instance at page 8, lines 34-35. The amendments to the claims and new Claim 12 do not introduce new matter.

The objection to the specification has been overcome by correcting the spelling of the word "weight" at page 1, line 36.

The rejection of Claims 1-11 for obviousness-type double patenting as being unpatentable over Claims 1-20 of US patent 6,723,737 has been overcome by the attached terminal disclaimer. The filing of a terminal disclaimer is not to be construed as an admission, estoppel or acquiescence. See *Quad Environmental Technology v. Union Sanitary District*, 20 USPQ2d 1392 (Fed. Cir. 1991) and *Ortho Pharmaceuticals Corp. v. Smith*, 22 USPQ2d 1119 (Fed. Cir. 1992).

The rejection of Claims 1-3 under 35 USC 112, 2nd paragraph in the recitation of "at least one member selected from the group consisting of adenosine 5'-triphosphate and adenosine 5'-monophosphate has been overcome by the cancellation of the term "at least one" from these claims.

The rejection of Claims 1-11 under 35 USC 112, 1st paragraph has been overcome by the cancellation of Claim 3.

With respect to the recitation concerning adenosine 5'-monophosphate, the specification is sufficient to teach those skilled in the art of the use thereof pursuant to the present invention. The specification discloses that the beneficial effect of the present invention is achieved by the cycling of adenosine and inorganic phosphate incorporation into liver ATP pools, the turnover of which supplies the adenosine precursor for expanded red blood cell ATP synthesis followed by release of red blood cell ATP into the blood plasma compartment and the continuous degradation of blood plasma ATP pools to adenosine and inorganic phosphate. For example, please see page 8, lines 14-25 of the present specification. Once aware of the present disclosure, person skilled in the art would appreciate that adenosine and inorganic phosphate or adenosine 5'-monophosphate or adenosine 5'-triphosphate are all effective in generating elevated levels of extracellular ATP, which in turn undergoes catalytic degradation to elevated levels of extracellular adenosine for the purposes of the present invention. For example, see US Patent 5,227,371 (copy enclosed) which discloses active agents, the administration of which result in elevated blood plasma levels (extracellular) of adenosine 5'-triphosphate, which in turn undergoes continuous rapid degradation to adenosine. Adenosine and inorganic phosphate, adenosine 5'-monophosphate or adenosine 5'-triphosphate, when administered to a host, produce elevated levels of blood plasma (extracellular) adenosine 5'-triphosphate, which is degraded to (elevated levels of) adenosine by ectoenzymatic catabolic activities present in the vascular bed as well as enzymatic activities present in the blood plasma. Thus, the enablement for adenosine 5'-triphosphate (ATP), which is disclosed and taught in a non-limiting fashion, would indicate to the skilled artisan that adenosine and inorganic phosphate or adenosine 5'-monophosphate (AMP) are likely to possess similar activities in obtaining weight loss in humans. The reason is that these active agents undergo in vivo metabolism similar to that of ATP, as disclosed and taught in US Patent 5,227,371, resulting in the elevation of blood plasma adenosine.

Furthermore, the fact that adenosine 5'-monophosphate as compared to adenosine is a better source of adenosine in vivo, has recently been confirmed by a clinical trial. The study started in October 2003, well after the PCT publication date of applicant's corresponding International application. A copy of the summary of the study purpose is enclosed (AMP as a Better Delivery System of Adenosine).

As demonstrated in U.S. Patent No. 5,227,371 and in the summary of a clinical trial of administration of AMP versus adenosine, the utilization of adenosine 5'-triphosphate in the

instant specification was in a non-limiting fashion. Moreover, with regard to combinations of ATP and adenosine A1 receptor antagonists, the examiner's attention is kindly directed to Dagnelie PC and Beijer S, Clinical Nutrition Abstract, Vol.22, Suppl. 1, page S61, 2003 (copy enclosed).

Moreover, it is well settled that to satisfy the enablement requirement, an application need not teach, and preferably omits, that which is well known in the art. How such a teaching is set forth, whether by the use of illustrative examples or by broad descriptive terminology, is of no importance since the specification which teaches how to make and use the invention in terms which correspond in scope to the claims must be taken as complying with the first paragraph of 35 U.S.C. 112 unless there is reason to doubt the objective truth of the statements relied upon therein for enabling support. See In re Marzocchi,, 439 F .2d 220, 223-24, 169 USPQ 367, 369-70 (CCPA 1971). The specifications to the instant application did not need to explicitly use adenosine 5'-monophosphate or adenosine and inorganic phosphate as adenosine generating agents ("adenosine active agents") since these activities were already well known in the art, although not for the claimed purposes.

As to quantitative aspects of the claims, no undue experimentation will be required of skilled artisans practicing the instant invention. For instance, person skilled in the art familiar with these aspects of administration of adenosine active agents, after publication of applicant's PCT application were able to then carry out clinical trials as demonstrated by the above mentioned Dagnelie PC and Beijer S Abstract and the AMP versus adenosine clinical trial. No undue experimentation will thus be required of skilled artisans practicing the instant invention.

In view of the above amendment, applicant believes the pending application is in condition for allowance.

Applicant believes no fee is due with this response. However, if a fee is due, please charge our Deposit Account No. 22-0185, under Order No. 21095-00008-US1 from which the undersigned is authorized to draw.

Dated: 7-24-06

Respectfully submitted,

By

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Conclusions: These data form the basis for a randomised controlled human trial to monitor the full efficacy of the synbiotic, paramount for understanding the composition of the microflora in IBS patients and developing a successful treatment strategy.

Topic: Malignancies

226-O. DOES CAFFEINE BLOCK THE FAVOURABLE EFFECTS OF ADENOSINE 5'-TRIPHOSPHATE (ATP) ON THE NUTRITIONAL STATUS OF CANCER PATIENTS?

P.C. Dagnelie, S. Beijer
Department of Epidemiology, Maastricht University, Maastricht, Netherlands

Rationale: A recent randomised clinical trial in 58 patients with advanced lung cancer revealed marked positive effects of regular ATP infusions on body weight, fat mass and fat-free mass [Agteresch HJ et al. JNCI 2000; 92: 321-8 and J Clin Oncol 2002;20: 371-8]. The effects of extracellular ATP are mediated by P1 and P2 receptors. Since caffeine is a well-known non-selective adenosine (P1) receptor antagonist, we tested the following hypothesis: 1. caffeine consumption has a negative effect on body weight, fat mass and fat-free mass in cancer patients; 2. the negative effect of caffeine is stronger in the ATP-treated patients.
Method: Data from the above-mentioned trial were reanalysed. Caffeine intake was calculated from 7-day food diaries at baseline, 8 and 16 wks. Time effects of caffeine intake were expressed as weight change (kg/4wks) per 100mg increase of caffeine intake (repeated analysis, SASProcMixed).
Results: In contrast with hypothesis 1, caffeine showed a significant ($p \leq 0.01$) positive relation with changes in weight, fat mass and fat-free mass over time in the control group (for weight: +0.30kg/4wks/100mg caffeine), especially for tea (+1.29kg/4wks/100mg, $p \leq 0.001$) but not for coffee (+0.23kg/100mg, NS). In the ATP group, this effect seemed less marked for total caffeine (difference with control group: -0.20kg/100mg, NS), coffee (-0.20kg, NS) and tea (-1.87kg, $p \leq 0.01$).
Conclusions: Hypothesis 1 was rejected: caffeine intake (especially from tea) was positively related with changes in weight, fat mass and fat-free mass over time in advanced cancer patients. Since patients were not randomised for caffeine intake, this association may have been caused by confounding. Hypothesis 2 was not rejected: In ATP-treated patients, the positive effect of caffeine was absent, or even opposite for tea, which argues for caffeine abstinence during ATP treatment.

227-P. GLUTAMINE ENHANCED NUTRITION IN GASTRIC CANCER PATIENTS

Z. Csepo[1], G. Vesztergombi[2], L. Harsanyi[1]
[1] *1st Department of Surgery,* [2] *Department of Radiology, Semmelweis University, Budapest, Hungary*

Rationale: The aim of our study was to evaluate 1) is there any connection between pre-operative nutritional state of patients with gastric malignancy and early post-operative complications, and 2) wether early post-operative immunonutrition can help to decrease the rate of these complications.
Method: The method was a prospective clinical trial on patients who underwent gastrectomy due to proved gastric cancer. In two groups the patients were checked for body mass index, weight loss rate, serum albumine level, serum lymphocyte count. In the early post-operative period the patients were followed for any kind of complications while in the first group (n=36) the patients received the normal post-operative jejunal feeding (Nutrison, Nutricia) and in the second group (n= 12) they received the same energy (25 kcal/body weigh) and protein containing diet with glutamine addition (Stresson, Nutricia).
Results: In the first group 17 patients had uneventful post-operative period, while 19 patients had complications. There were patients with more than one complication which resulted that 9 surgical complication and 22 non-surgical complications were detected. The surgical complications devided to 4 septic and 3 non-septic wound disruptions and two anastomosis leakage. The non-surgical complications contained 3 septic ones (pneumony). In the second group 10 patients cured without complication and 2 patients had wound suppuration and/or anastomosis leakage. The post-operative hospital stay was nearly 20% longer in the first group (14.8 days to 11.7 days), with the connection of the costs as well.
Conclusions: Although our patients number is low, we conclude that the early jejunal feeding with glutamine enriched nutrient can give a good chance to defend early postoperative complications and shorten the hospital stay and costs.

228-P. QUALITY OF LIFE UNDER HOME PARENTERAL NUTRITION WITH SPECIAL EMPHASIS ON CANCER PATIENTS

P. Thul[1], K. Bauer[2]
[1] *Clinic of General, Visceral, Vascular and Thoracic Surgery, Campus-Charité Mitte, Berlin, Germany,* [2] *Department of Surgery, Kantonsspital, Schaffhausen, Switzerland*

Rationale: This study examined the quality of life (LQ) in patients under long-term (at least 6 months) home parenteral nutrition (HPN), mainly for cancer patients.
Method: 80 patients received over at least 6 month HPN and were followed for a maximum of 41 months. Because of progressive disease 46 patients could be interviewed only twice, the remaining 34 patients (13 women, 21 men) answered the EORTC QLQ-C30 questionnaire (Version 2.0) 6 to 27 times in addition to 10 questions specific for HPN.
Results: The results for LQ were differentiated between patients with malignant (MD) and benign disease (BD) and with relapse of tumor or not. At the beginning of the examination period patients with BD showed for physical ability (PA) mean values of 53.3 out of 100 points of the EORTC QLQ-C30 scale going down to 32.5 at the end. Social activities resulted in 29.9 vs 41,9 points respectively. Patients with MD (relapse-free) showed values from 54,8 to 54,1, patients with tumor-relapse 69,1 to 61,9 points concerning PA. Similar result were found for the symptom scales. Patients with BD complained more about pain, nausea, loss of appetite, fatigue and fear. A weight gain or constant weight could be measured in 24 patients. 10 patients lost weight because of their progressive disease.
Conclusions: Inspite of progressive disease LQ stabilized or improved in 41% of patients under HPN. 30% of patients were unchanged over at least 6 months. Since under HPN life activity is only marginally changed, indication should be made as early as possible. Regarding the function scales of EORTC QLQ-C30, patients with MD profit more from therapy.

229-P. THE AGREEMENT BETWEEN MEASURED AND PREDICTED RESTING ENERGY EXPENDITURE IN PATIENTS WITH PANCREATIC CANCER - A PILOT STUDY

J.D. Bauer[1], M.M. Reeves[2], S. Capra[3]
[1] *Nutrition Program, The Wesley Research Institute, Toowong,* [2] *Centre for Health Research, Queensland University of Technology, Brisbane,* [3] *School of Health Sciences, University of Newcastle, Callaghan, Australia*

Rationale: To compare measured resting energy expenditure (REE) to predicted REE from eight published equations in a sample of patients with pancreatic cancer.
Method: Eight pancreatic cancer patients (age 60±5y; BMI 24.4±3.2kg/m²; 5M:3F); four with multiple measurements at least four weeks apart, following changes in weight and body composition (total of 15 measurements). REE measured by indirect calorimetry (VMax229, Sensormedics) and predicted from eight published prediction equations; body composition estimated by deuterium oxide dilution technique; paired t-tests and Bland & Altman analysis.
Results: At the group level, there was no significant difference between mean measured (6557 ± 1131 kJ/d) and predicted REE from the Harris-Benedict (no injury factor), Schofield, Owen, Mifflin, Wang and Cunningham equations and the 20 kcal/kg ratio method. The Harris-Benedict x 1.3 resulted in a significantly higher mean predicted REE compared to mea-

Vol. 22 Supplement 1, 2003

Conclusions: These data forms the basis for a randomised controlled human trial to monitor the full efficacy of the synbiotic, paramount for understanding the composition of the microflora in IBS patients and developing a successful treatment strategy.

Topic: Malignancies

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Rationale: A recent randomised clinical trial in 58 patients with advanced lung cancer revealed marked positive effects of regular ATP infusions on body weight, fat mass and fat-free mass [Agteresch HJ et al. JNCI 2000; 92: 321-8 and J Clin Oncol 2002;20: 371-8]. The effects of extracellular ATP are mediated by P1 and P2 receptors. Since caffeine is a well-known non-selective adenosine (P1) receptor antagonist, we tested the following hypothesis: 1. caffeine consumption has a negative effect on body weight, fat mass and fat-free mass in cancer patients; 2. the negative effect of caffeine is stronger in the ATP-treated patients.
Method: Data from the above-mentioned trial were reanalysed. Caffeine intake was calculated from 7-day food diaries at baseline, 8 and 16 wks. Time effects of caffeine intake were expressed as weight change (kg/4wks) per 100mg increase of caffeine intake (rep.meas.analysis, SASProcMixed).
Results: In contrast with hypothesis 1, caffeine showed a significant ($p \leq 0.01$) positive relation with changes in weight, fat mass and fat-free mass over time in the control group (for weight: +0.30kg/4wks/100mg caffeine), especially for tea (+1.29kg/4wks/100mg, $p \leq 0.001$) but not for coffee (+0.23kg/100mg, NS). In the ATP group, this effect seemed less marked for total caffeine (difference with control group: -0.20kg/100mg, NS), coffee (-0.20kg, NS) and tea (-1.87kg, $p \leq 0.01$).
Conclusions: Hypothesis 1 was rejected: caffeine intake (especially from tea) was positively related with changes in weight, fat mass and fat-free mass over time in advanced cancer patients. Since patients were not randomised for caffeine intake, this association may have been caused by confounding. Hypothesis 2 was not rejected: In ATP-treated patients, the positive effect of caffeine was absent, or even opposite for tea, which argues for caffeine abstinence during ATP treatment.

227-P. GLUTAMINE ENHANCED NUTRITION IN GASTRIC CANCER PATIENTS

Z. Csapo[1], G. Vesztergombi[2], L. Harsanyi[1]
[1]*1st Department of Surgery,* [2]*Department of Radiology, Semmelweis*

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228-P. QUALITY OF LIFE U NUTRITION WITH SPECIAl PATIENTS

P. Thul[1], K. Bauer[2]
[1]*Clinic of General, Visceral, Vas Charit Mitte, Berlin, Germany, Schaffhausen, Switzerland*

Rationale: This study examined long-term (at least 6 months) ho cancer patients.
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229-P. THE AGREEMENT B PREDICTED RESTING ENE WITH PANCREATIC CANCI

J.D. Bauer[1], M.M. Reeves[2], S. (
...ram, The Wesley

AMP as a Better Delivery System of Adenosine

This study is currently recruiting patients.
Verified by Vanderbilt University September 2005

Sponsored by: **Vanderbilt University**
Information provided by: Vanderbilt University
ClinicalTrials.gov Identifier: NCT00179010

Purpose

Adenosine and AMP are substances normally present in the body. Adenosine is also given for the treatment of some heart rhythm problems and may be used to reduce heart damage during heart attacks. The problem in using adenosine is that it is taken up by cells and, therefore, very little of the adenosine we give by vein or in the artery actually reaches the tissue. We propose to use AMP as a way to improve delivery of adenosine. AMP is inactive by itself, but is converted to adenosine in tissue. We hope that by giving AMP we will increase levels of adenosine in tissue. To see if this is true, we will give either adenosine or AMP into the forearm artery while we measure how much adenosine reaches the forearm tissue.

Condition	Intervention	Phase
Ischemia	Drug: Adenosine Drug: Adenosine Mono Phosphate (AMP)	Phase II

MedlinePlus related topics: Vascular Diseases
Genetics Home Reference related topics: Vascular Diseases

Study Type: Interventional
Study Design: Diagnostic, Randomized, Single Blind, Active Control, Crossover Assignment, Efficacy Study

Official Title: Use of AMP to Improve Tissue Delivery of Adenosine

Further study details as provided by Vanderbilt University:
Primary Outcomes: Interstitial adenosine levels; Forearm blood flow; Heart rate; Blood pressure
Secondary Outcomes: Symptoms
Expected Total Enrollment: 100

Study start: October 2003
Last follow-up: April 2005

Eligibility

Ages Eligible for Study: 18 Years - 65 Years, Genders Eligible for Study: Both

Accepts Healthy Volunteers

Criteria

Inclusion Criteria:

- Healthy volunteers
- Age 18-65
- Non smokers

Exclusion Criteria:

- Smokers
- Any chronic disease

Location and Contact Information

Please refer to this study by ClinicalTrials.gov identifier NCT00179010

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Alfredo Gamboa, M.D., Sub-Investigator
Cyndya Shibao, M.D., Sub-Investigator
Andrew Ertl, Ph.D., Sub-Investigator
Andre Diedrich, M.D., Ph.D, Sub-Investigator

Study chairs or principal investigators

Italo Biaggioni, M.D., Principal Investigator, Vanderbilt University

More Information

Study ID Numbers: 030371
Last Updated: December 8, 2005
Record first received: September 13, 2005
ClinicalTrials.gov Identifier: NCT00179010
Health Authority: United States: Food and Drug Administration
ClinicalTrials.gov processed this record on 2006-05-01

US005227371A

United States Patent [19]

Rapaport

[11] Patent Number: 5,227,371

[45] Date of Patent: Jul. 13, 1993

[54] UTILIZATION OF ADENINE NUCLEOTIDES AND/OR ADENOSINE AND INORGANIC PHOSPHATE FOR ELEVATION OF LIVER, BLOOD AND BLOOD PLASMA ADENOSINE 5'-TRIPHOSPHATE CONCENTRATIONS

[76] Inventor: Eliezer Rapaport, 142 Payson Rd., Belmont, Mass. 02178

[21] Appl. No.: 400,547

[22] Filed: Aug. 31, 1989

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 223,503, Jul. 25, 1988, Pat. No. 5,049,372, which is a continuation-in-part of Ser. No. 397,897, Jul. 13, 1982, Pat. No. 4,880,918.

[51] Int. Cl.[5] A61K 31/70

[52] U.S. Cl. 514/46; 514/47

[58] Field of Search 514/45, 46, 47, 48

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(List continued on next page.)

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Attorney, Agent, or Firm—Pollock, Vande Sande & Priddy

[57] ABSTRACT

Administration of adenine nucleotides to a host is followed by their rapid degradation to adenosine and inorganic phosphate which promote increases in liver ATP pools. The turnover of expanded liver ATP pools supply the adenosine precursor for the subsequent expansions of red blood cell (total blood) and blood plasma (extracellular) ATP pools. Thus, the administration of AMP, ATP or their degradation products adenosine and inorganic phosphate to a host, achieve the beneficial increases in liver, total blood and blood plasma ATP levels.

45 Claims, No Drawings

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UTILIZATION OF ADENINE NUCLEOTIDES AND/OR ADENOSINE AND INORGANIC PHOSPHATE FOR ELEVATION OF LIVER, BLOOD AND BLOOD PLASMA ADENOSINE 5'-TRIPHOSPHATE CONCENTRATIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation-in-part of co-pending U.S. application Ser. No. 07/223,503 filed Jul. 25, 1988, now U.S. Pat. No. 5,049,372, entitled, "Anticancer Activities in a Host by Increasing Blood and Plasma Adenosine 5'-Triphosphate (ATP) Levels", which in turn is a continuation-in-part of U.S. patent application Ser. No. 06/397,897 filed Jul. 13, 1982, now U.S. Pat. No. 4,880,918, entitled, "Arrest and Killing of Tumor Cells by Adenosine 5'-Diphosphate and Adenosine 5'-Triphosphate", disclosures of which are incorporated herein by reference.

DESCRIPTION

1. Technical Field

The present invention is concerned with the use of adenine nucleotides and/or adenosine and inorganic phosphate separately or in combinations for the purpose of elevating liver, blood (total cellular) and blood plasma (extracellular) levels of adenosine 5'-triphosphate. Because of the extremely rapid degradation of adenine nucleotides; adenosine 5'-monophosphate (AMP), adenosine 5'-diphosphate (ADP) or adenosine 5'-triphosphate (ATP) in the vascular bed by enzymatic activities present in blood plasma and on the membrane of blood cells or vascular endothelium (ectoenzymes). it is widely assumed that the physiological and pharmacological activities of adenine nucleotides after their administration into animals or humans are due to the activity of their degradation product, namely adenosine. The present invention demonstrates that administration of adenine nucleotides (AMP, ADP and/or ATP) or adenosine and inorganic phosphate, which are the degradation products of adenine nucleotides in the peritoneal cavity or in the systemic circulation, into a host, yields elevated levels of liver, blood and blood plasma ATP pools. This finding was totally unexpected and the mechanism accounting for the formation of elevated liver, blood and blood plasma ATP pools has been established and is outlined in the present invention. Since extracellular ATP was shown to produce beneficial effects on a variety of cells and tissues in vitro as ATP and not as one of its degradation products, the present invention offers a variety of beneficial effects in vivo in a host. The reason being that this invention establishes an unexpected process for elevating extracellular (blood plasma) ATP concentrations in such a host. The elevated liver, blood and blood plasma ATP pools are not related to the ATP which is administered to the host since the administered ATP undergoes extremely rapid degradation to adenosine and phosphate. As is demonstrated in this invention, substantial increases in liver, blood and blood plasma ATP pools can also be achieved by the administration of adenine nucleotides other than ATP, or by the administration of their degradation products, namely adenosine and phosphate.

2. Background Art

Extracellular ATP was shown to have a variety of physiological effects on individual cells and tissues. Extracellular ATP was demonstrated to have beneficial

physiological effects on the regulation of vascular tone [Burnstock, G. and Kennedy, C. A dual function for adenosine 5'-triphosphate in the regulation of vascular tone. Circul. Res. 58, 319-330 (1986); Kennedy, C. and Burnstock, G. Evidence of two types of P_2-purinoceptor in longitudinal muscle of the rabbit portal vein. Eur. J. Pharmacol. 111, 49-56 (1985)], muscle contraction [Burnstock, G. Purinergic nerves. Pharmacol. Rev. 24, 509-581 (1972)], cardiovascular function [Berne, R. M. The role of adenosine in the regulation of coronary blood flow. Circul. Res. 47, 807-813 (1980)], neurotransmission [Sneddon, P. and Burnstock, G. ATP as a cotransmitter in rat tail artery. Eur. J. Pharmacol. 106, 149-152 (1984)], and platelet aggregation [Holmsen, H. Platelet metabolism and activation. Sem. Hematology 22, 219-240 (1985)]. In addition, elevated levels of extracellular ATP were shown to have favorable effects on a variety of blood cells, among then lymphoid cells [Ikehera, S., Pahwa, R. N., Lunzer, D. G., Good, R. A. and Modak, M. J. Adenosine 5'-triphosphate-(ATP) mediated stimulation and suppression of DNA synthesis in lymphoid cells. J. Immunol. 127, 1834-1838 (1981)], thymocytes [El-Moatassin, C., Dornand, J. and Mani, J. C. Extracellular ATP increases cytosolic free calcium in thyocytes and initiates the blastogenesis of the phorbol 12-myristate 13-acetate-treated medullary population. Biochem. Biophys. Acta 927, 437-444 (1987)], and neutrophils (Kuroki, M. and Minakami, S. Extracellular ATP triggers superoxide production in human neutrophils. Biochem. Biophys. Res. Commun. 162, 377-380 (1989)]. Recently, U.S. patent applications by Rapaport (Ser. No. 397, 897,now U.S. Pat. No. 4,880,918 and Ser. No. 223,503, now U.S. Pat. No. 5,049,372) have demonstrated that extracellular levels of ADP and/or ATP inhibit tumor growth and that the administration of adenine nucleotides (AMP, ADP or ATP) into a host result in the elevation of extracellular blood plasma levels of ATP which in turn inhibit tumor growth in a host and also ameliorate cancer cachexia by inhibiting host weight loss in tumor-bearing hosts (See also Rapaport, E. Experimental cancer therapy in mice by adenine nucleotides. Eur. J. Cancer & Clin. Oncol. 24, 1491-1497 (1988); Rapaport, E. and Fontaine, J. Anticancer activities of adenine nucleotides in mice are mediated through expansion of erythrocyte ATP pools. Proc. Natl. Acad. Sci. USA 86, 1662-1666 (1988)).

Because of the extremely rapid breakdown of ATP and other adenine nucleotides to adenosine after their administrations into animals and humans, it has been widely assumed that the beneficial pharmacological effects of ATP or other adenine nucleotides are due to their degradation product, adenosine. In a recent review [Gordon, J. L. Extracellular ATP: effects, sources and fate. Biochem. J. 233, 309-319 (1986)], the author states that "It has been known since 1950 that a bolus of ATP is virtually all removed by a single passage through the lung" and that "there are, apparently, three separate enzymes that sequentially catabolize ATP→ADP→AMP→Adenosine" (page 316 under subsection Metabolism). In another recent review [Flacke, W. E. Adenosine and adenosine triphosphate for acute blood pressure control. Seminars in Anesthesia 7, 216225 (1988)] the author states that "ATP is rapidly broken down to adenosine after systemic administration, after I.V. administration mainly while transiting the lungs, and the hypotensive effect parallels arterial adenosine concentrations" (page 217).

In light of the many favorable effects exerted by extracellular ATP in vitro on a variety of cells and tissues and the widely accepted knowledge that ATP and other adenine nucleotides are rapidly degraded immediately after their administration into animals or human hosts to their catabolic product adenosine, this invention was completely unexpected and existing art essentially taught away from it. This invention demonstrates that in vivo administered adenine nucleotides or adenosine and inorganic phosphate (but not adenosine alone) or mixtures of these compounds yield a sustained "secondary wave" of extracellular blood plasma ATP levels resulting from the immediate rapid degradation of the administered adenine nucleotides to adenosine and inorganic phosphate which in turn promote the expansion of liver ATP pools followed by expansion of red blood cell (RBC) ATP pools and the release of micromolar levels of ATP from these RBCs into the extracellular (blood plasma) compartment.

Although U.S. patent application Ser. No. 223,503 claims utilization of AMP and ATP for the purpose of elevating total blood (cellular) and blood plasma (extracellular) pools of ATP, the invention outlined in the current application demonstrates that the degradation products of any adenine nucleotide (e.g. AMP, ADP or ATP) namely adenosine and inorganic phosphate (but not adenosine alone) promote the effective elevation of total blood and blood plasma ATP pools in experimental animals which were used to demonstrate this invention in a non-limiting fashion. This process differs from the direct introduction of AMP or ATP by the unexpected mechanistic aspects which are now established and which are outlined in the Summary of the Invention section. Furthermore, this invention is significant for the purpose of elevating liver, blood and plasma ATP pools because the most abundant commercial source of ATP which is yeast, contains mixtures of adenine nucleotides along with adenosine and inorganic phosphate [Krallish, I. L., Damberga B. E. and Beker, M. J. State of adenosine phosphates during dehydration of yeast. Applied Microbiology Biotechnology 31, 194-199 (1989)]. It would be of great commercial advantage not to have to purify these mixtures of adenine nucleotides into their individual components before its utilization for the purpose of expanding liver, blood and plasma ATP pools in a host.

SUMMARY OF THE INVENTION

The present invention demonstrates by the use of mice as experimental hosts in a non-limiting fashion, that the administration of AMP, ADP, ATP, or adenosine and inorganic phosphate (but not adenosine alone) results in the unexpected expansion of liver, red blood cell (RBC) ATP pools and blood plasma ATP levels. These results were demonstrated in experimental animals under normal pathophysiological conditions. In order to demonstrate the invention, high specific radioactivity, radioactively-labeled precursors [^{3}H] or [^{3}H, α-^{32}P]ATP were administered intraperitoneally into mice in 2 ml of saline or in 2 ml of 35 mM of adenosine, AMP, ATP or adenosine and inorganic phosphate in saline. The results show that adenosine, AMP, ATP or adenosine and inorganic phosphate expand the total liver ATP pools by 2-3 fold as compared to saline or no treatment. However, only AMP, ATP or adenosine and inorganic phosphate yielded expansions of RBC ATP pools and increases in blood plasma ATP levels. Furthermore, the specific radioactivities of liver [^{3}H]ATP pools were similar to the specific radioactivities of RBC [^{3}H]ATP pools after AMP, ATP or adenosine and inorganic phosphate injections, suggesting that the turnover of expanded liver [^{3}H]ATP pools provide the [^{3}H]adenosine precursor which is needed for the enhanced synthesis of RBC ATP pools. Mature RBCs cannot synthesize ATP de novo and require salvage precursors for ATP synthesis [Lerner, M. H. and Lowy, B. A. The formation of adenosine in rabbit liver and its possible role as a direct precursor of erythrocyte adenine nucleotides. J. Biol. Chem. 249, 959-966 (1974)]. Injections of adenosine alone, contrary to injections of adenosine and inorganic phosphate, resulted in expansions of total liver ATP pools without any effects on RBC ATP pools or plasma ATP levels. The specific radioactivity of RBC [^{3}H]ATP pools was vastly different from the specific radioactivity of liver [^{3}H]ATP pools after injections of adenosine or saline containing high specific radioactivity labeled precursors. It is important to note that all the precursors, namely, adenosine, AMP, ATP or high specific radioactivity [^{3}H]ATP are incorporated into liver ATP pools as adenosine or [^{3}H]adenosine respectively. Ectoenzymatic catabolic activities present in the peritoneal cavity and in the vascular bed as well as soluble enzymatic activities present in blood plasma, actively catalyze the degradation of adenine nucleotides to adenosine and inorganic phosphate which was shown to be the combination that promotes the expansion of liver ATP pools followed by the expansions of RBC and blood plasma ATP pools. By the utilization of a doubly labeled [^{3}H,α^{32}P]ATP as a high specific radioactivity label, the fate of both the ^{3}H-labeled adenosine moiety and the ^{32}P-labeled inorganic phosphate moiety could be followed. It was shown by this invention that the administered adenine nucleotides are rapidly degraded to adenosine and inorganic phosphate and that the combination of adenosine and inorganic phosphate is required for the expansion of RBC ATP pools. Adenosine alone is sufficient to expand the total liver ATP pools but the inorganic phosphate, which is either a degradation product of adenine nucleotides along with adenosine or is administered along with adenosine, is essential for the expansion of RBC ATP pools. This expansion of RBC ATP pools occurs after the circulating RBCs take-up the adenosine needed for the enhanced salvage synthesis of ATP in RBCs. The circulating RBCs take-up the adenosine, which is produced from the turnover of the expanded liver ATP pools, in the hepatic sinusoids. This fact was demonstrated by the similarity in the specific radioactivities of liver [^{3}H]ATP pools and RBC [^{3}H]ATP pools which lasted for several hours, indicating that these pools effectively mix via a common precursor. When liver [^{3}H]ATP pools were radioactively labeled along with the administration of adenosine or saline, which do not cause expansion of RBC ATP pools, although adenosine treatment does expand the liver ATP pools, the specific radioactivities of liver and RBC [^{3}H]ATP pools were vastly different. The expanded RBC ATP pools are slowly released into the blood plasma (extracellular) compartment in micromolar amounts as was shown by the increases in blood plasma ATP levels, which were determined by bioluminometry and specific radioactivity after expansions of RBC ATP pools achieved by the administration of AMP, ATP or adenosine and inorganic phosphate. The slow continuous release of micromolar amounts of ATP from RBCs containing expanded ATP pools, al-

lows the maintenance of elevated blood plasma ATP pools in spite of the rapid turnover of these pools due to the catabolic activities present in the vascular bed, which effectively degrade these extracellular ATP pools.

BEST AND VARIOUS MODES FOR CARRYING OUT THE INVENTION

The present invention demonstrates in mice as experimental animals utilized in a non-limiting fashion that administration of adenosine 5'-monophosphate (AMP), adenosine 5'-triphosphate (ATP) or mixtures of adenosine and inorganic phosphate (which is any inorganic phosphate salt) result in substantial increases in liver ATP pools, red blood cell (RBC) ATP pools and blood plasma (extracellular compartment) ATP pools. The term pool denotes a steady state concentration which is the result of a specific rate of synthesis and degradation. The significance of this invention is reflected by the facts that many favorable and beneficial (to human hosts) physiological functions are affected by increases in liver, RBCs and blood plasma ATP pools and the commercial availability of mixtures of adenine nucleotides or adenosine and inorganic phosphate rather than pure isolated nucleotides (as reviewed in the Background Art Section). This invention offers therefore new and distinct advantages. The treatment of a host with AMP, ATP, adenosine and inorganic phosphate or mixtures of adenine nucleotides for the purpose of expanding liver, RBC and blood plasma ATP pools can be employed in a pharmaceutically acceptable salt form and can also be employed in a variety of conventional pharmaceutical preparations. These preparations can contain organic or inorganic material suitable for internal administration. The high solubility of AMP and/or ADP and/or ATP salts and/or adenosine and phosphate salts in isotonic aqueous solutions of sodium chloride enable administration of these agents in the form of injection or infusion of single or multiple doses. The injection or infusion can be intraperitoneal, intravenous, or intra-arterial. AMP and/or ADP and/or ATP and/or adenosine and phosphate salts are also suitable for oral, enteral, or topical application when employed with conventional organic or inorganic carrier substances. The effective doses should be in the range of about 1-1,000 mg/kg of body weight per 24 hours for oral or topical administration and 0.01-500 mg/kg of body weight per 24 hours for injections. Intravenous, intraperitoneal, or intraarterial infusions of AMP and/or ADP and/or ATP and/or adenosine and phosphate salts in a suitable salt form is preferably administered at a rate of about 0.001-10 mg/kg of body weight per minute. The delivery of these agents can be performed using a variety of drug delivery systems including, but not limited to, pumps or liposomes.

Examples of inorganic phosphates are sodium phosphate, potassium phosphate and phosphoric acid. The pH of any solution employed containing the phosphate is usually adjusted, if necessary, to about 6.0 to about 7.5 and preferably to about 6.2 to about 7.0 by the addition of a base such as sodium hydroxide. Usually at least about 1 equivalent of phosphate per adenosine is employed.

The following experiments demonstrate the present invention in a non-limiting fashion. Blood (0.25 ml) was collected into 1-ml syringes (26-gauge needles) containing either citrate/dextrose (0.05 ml of 93 mM sodium citrate/7 mm citric acid/140 mM dextrose, pH 6.5) or

sodium heparin (0.05 ml of 3 units of sodium heparin in saline) from the inferior vena cava. Mice were anesthetized with ether during the procedure. Plasma or conditioned Hanks' balanced salt solution (HBSS) from the incubation of isolated RBCs was prepared by centrifugation of whole blood or RBCs, respectively, in a Beckman Microfuge (30 sec at 8000×g), and samples of 100 µl were added to 1 ml of ice-cold 7% (wt/vol) trichloroacetic acid. RBCs were prepared by centrifugation of whole blood (1500×g for 5 minutes at 4° C.), and removal of plasma and the buffy coat was followed by a wash of the pelleted RBCs (from 250 µl of whole blood) in 5 ml of ice-cold HBSS. After centrifugations, the RBC pellet was resuspended in a volume of HBSS to yield the original hematocrit (percent of RBC volume in the whole blood). Aliquots of 20 µl of RBC suspensions or whole blood were added to 1 ml of ice-cold 7% trichloroacetic acid.

Extraction of acid-soluble nucleotides and determinations of ATP levels by luminometry followed published procedures [Rapaport, E. Experimental cancer therapy in mice by adenine nucleotides. Eur. J. Cancer & Clin. Oncol. 24, 1491-1497 (1988)]. For the determination of total ATP pools and specific radioactivities of [^{3}H]ATP in liver, RBC and blood plasma, blood (0.25 ml) was collected from the inferior vena cava into 1 ml syringes containing 0.05 ml of citrate-dextrose as described previously at a variety of time points after the intraperitoneal injections of AMP, ATP, adenosine and phosphate salts or adenosine alone in saline or injections of saline alone containing in all cases high specific radioactivity [^{3}H]ATP or [^{3}H,α-^{32}P]ATP as radioactive precursors. Mice were anesthetized with ether during the blood and tissue removing procedure and immediately after the removal of the blood by one person, another person excised a small portion of the liver (250-400 mg) utilizing in situ freeze-clamping with aluminum plates precooled in liquid nitrogen [as described in Rapaport, E. and Zamecnik, P. C. Incorporation of adenosine into ATP:Formation of compartmentalized ATP. Proc. Natl. Acad. Sci. USA 73, 3122-3125 (1976)]. The frozen tissue was pulverized in a mortar at solid carbon dioxide temperatures and extracted in 10 ml of ice-cold 7% trichloracetic acid. Analyses of total liver, RBC and blood plasma ATP pools were performed by bioluminometry as described previously. Conversions of ATP levels to molar concentrations are based on weights of the frozen liver portions or total volume of blood in case of RBCs. The specific radioactivities of liver and RBC [^{3}H,α-^{32}P]ATP pools and blood plasma [^{3}H]ATP levels were determined by correlation of total radioactivity with total pool size. Total radioactivities of the ATP pools were determined by thin layer chromatography and total pool sizes were determined by bioluminometry. Determination of total radioactivity in blood plasma [^{3}H]ATP required two dimensional thin layer chromatography [according to Bochner, B. R. and Ames, B. N. Complete analysis of cellular nucleotides by two-dimensional thin layer chromatography. J. Biol Chem. 257, 9759-9769 (1982)].

Utilizing radioactively labeled precursors one is able to follow the phosphorylated adenosine derivatives after single i.p. injections of 2 ml of 35 mM adenosine, AMP or ATP. The results reported in Table 1 demonstrate that adenosine, AMP or ATP expand the total liver ATP pools by 2-3 fold as compared to saline treatment. However, only AMP and ATP yielded expansions of RBC ATP pools and increases in blood plasma

ATP levels. Furthermore, the specific radioactivities of liver ^{3}H]ATP pools were similar to the specific radioactivities of RBC [^{3}H]ATP pools after AMP or ATP injections, suggesting that the turnover of expanded liver [^{3}H]ATP pools provide the [^{3}H]adenosine precursor which is needed for the expanded synthesis of RBC ATP pools. Mature RBCs cannot synthesize ATP de novo and require salvage precursors for ATP synthesis as discussed earlier. Injections of adenosine resulted in expansions of total liver ATP pools without any effects on RBC ATP pools or plasma ATP levels. The specific radioactivity of RBC [^{3}H]ATP pools was vastly different from the specific radioactivity of liver [^{3}H]ATP pools after injections of adenosine or saline as control (Table 1). It is important to note that all the precursors, namely, adenosine, AMP, ATP or high specific radioactivity [^{3}H]ATP are incorporated into liver ATP pools as adenosine or [^{3}H]adenosine respectively. Ectoenzymatic catabolic activities present in the peritoneal cavity and in the vascular bed as well as enzymatic activities present in blood plasma, actively catalyze the degradation of ATP to adenosine.

The incorporation of a radioactive precursor into liver and RBC [^{3}H]ATP pools after i.p. injections of 2 ml of 35 mM ATP was followed as a function of time (Table 2). The similarity between the specific radioactivities of liver and RBCs [^{3}H]ATP pools was maintained throughout the expansion of the total ATP pools in both the liver (3 to 7 mM) and RBCs (0.6 to 1.7 mM) during the first two hours after injections (Table 2). Only at later times (3–4 hours after injection) did the specific radioactivity of liver [^{3}H]ATP decline at the expense of the increases in the specific radioactivity and size of the RBC [^{3}H]ATP pools (Table 2).

adenosine and inorganic phosphate. the $^{32}P/^3H$ ratios would have remained constant and would not have been proportionally reduced by the phosphate moieties of AMP and ATP. The phosphate groups of AMP or ATP are not necessary for the expansion of total liver ATP pools since adenosine alone was demonstrated to achieve this expansion. Inorganic phosphate however is required for the expansion of RBC ATP pools since 2 ml of 35 mM adenosine along with 105 mM of inorganic phosphate produce results similar to those achieved with 2 ml of 35 mM ATP (Table 3).

The data discussed above lead to the following conclusions:

1. Administrations of adenine nucleotides into a host yield expansions of total liver ATP pools, expansions of RBC ATP pools and blood plasma ATP pools.
2. The administered adenine nucleotides are degraded to adenosine and inorganic phosphate before their promoted expansions of liver, RBC and blood plasma ATP pools.
3. The degradation of adenine nucleotides to adenosine and inorganic phosphate is rapid and occurs in the peritoneal cavity as well as in the vascular bed.
4. A combination of adenosine and inorganic phosphate is needed for the expansions of RBC and blood plasma ATP pools whereas adenosine alone is sufficient for the expansion of liver ATP pool.
5. A mixture of adenosine and inorganic phosphate either administered as such or produced in situ after the administration of adenine nucleotides is necessary and sufficient for achieving the useful expansions of RBC ATP pools and of blood plasma ATP pools.

TABLE 1

Mouse (CB6F$_1$) liver and RBC [^{3}H]ATP pools and specific radioactivities after i.p. injections of [^{3}H]ATP in saline, adenosine, AMP or ATP*

Compound Administered	Liver ATP+ mM	Liver ATP cpm/nmol	RBCs ATP+ mM	RBCs ATP cpm/nmol	Blood Plasma ATP+ μM	Blood Plasma ATP cpm/nmol
Saline	2.78	1543	0.69	2644	0.71	3955
Adenosine	6.86	3693	0.67	2154	0.93	2637
AMP	7.63	2864	1.17	2725	1.53	3293
ATP	8.29	2196	1.52	2340	1.78	2819

*Mice (CB6F$_1$, males, 9 weeks old) were injected i.p. with 2 ml of 500 μCi of [^{3}H]ATP (30 Ci/mmol specific radioactivity) in saline, 35 mM adenosine, 35 mM AMP or 35 mM ATP and 2–2.5 hours later the animals were anesthetized and analyzed as described in the text. Data represents the average of two experiments.
+Total ATP pools determined by bioluminometry.
Specific radioactivities were determined by the correlation of cpm in [^{3}H]ATP pools which were determined by one or two dimensional thin layer chromatography with the actual size of the [^{3}H]ATP pool determined by bioluminometry.

Further studies of the metabolic fate of i.p. injected adenine nucleotides utilizing [^{3}H,α-^{32}P]ATP as the radioactive precursor show the following (Table 3). Both the phosphate and adenosine moieties of AMP or ATP are incorporated into liver ATP pools and since the phosphate groups of AMP or ATP successfully dilute the ^{32}P radioactive label, the resulting liver [^{3}H,α-^{32}P]ATP pools possess progressively lower $^{32}P/^3H$ ratios in proceeding from adenosine to AMP to ATP as i.p. precursors. The fact that the phosphate groups of AMP and ATP proportionally reduce the $^{32}P/^3H$ ratios in liver [^{3}H,α-^{32}P]ATP pools as compared to saline or adenosine injections with [^{3}H,α-^{32}P]ATP as the radioactive label (Table 3), proves the degradation of [^{3}H,α-^{32}P]ATP as well as AMP or ATP to adenosine and phosphate moieties prior to incorporation into liver ATP pools. If [^{3}H,α-^{32}P]ATP as well as AMP or ATP were incorporated en bloc without prior degradation to

TABLE 2

Mouse (CB6F$_1$) liver and RBC [^{3}H]ATP pools and specific radioactivities after i.p. injections of 2 ml of 35 mM [^{3}H]ATP*

Time After injection. min	Liver ATP+ mM	Liver ATP cpm/nmol	RBCs ATP+ mM	RBCs ATP cpm/nmol
No injection	3.16	—	0.60	—
15	4.24	1743	0.93	1612
30	4.05	2408	0.99	2424
60	7.09	2766	1.31	2671
120	6.13	2815	1.73	3078

TABLE 2-continued

Mouse ($CB6F_1$) liver and RBC [^{3}H]ATP pools and specific radioactivities after i.p. injections of 2 ml of 35 mM [^{3}H]ATP*

Time After injection, min	Liver ATP+ mM	Liver ATP cpm/nmol	RBCs ATP+ mM	RBCs ATP cpm/nmol
240	8.25	2513	2.39	3564

*Mice ($CB6F_1$ males, 8 weeks old) were utilized. All the experimental procedures are outlined in the text and in the footnotes to Table 1. Data represents the average of two experiments.
+Total pool size.
Specific radioactivity.

TABLE 3

Mouse (athymic nu/nu) liver and RBC [^{3}H, -^{32}P]ATP pools and specific radioactivities after i.p. injections of [^{3}H, α-^{32}P]ATP in saline, adenosine, AMP or ATP*

Compound Administered	Liver ATP+ mM	Liver ATP ^{3}H-cpm/nmol	Liver ATP ^{32}p/^{3}H	RBCs ATP+ mM	RBCs ATP ^{3}H-cpm/nmol	RBCs ATP ^{32}p/^{3}H
Saline	2.93	1961	2.5	0.73	2874	2.5
Adenosine	6.42	2513	2.1	0.65	1063	0.4
AMP	9.10	1743	1.4	1.33	1555	0.4
ATP	9.46	2376	0.6	1.64	2240	0.5
Adenosine + Pi	7.71	1764	0.8	1.61	1899	0.6

*Mice (athymic nu/nu females, 9 weeks old) were injected i.p. with 2 ml of 500 μCi of [^{3}H]ATP (30 Ci/mmol) and 250 μCi [α-^{32}P]ATP (36 Ci/mmol) in saline, 35 mM adenosine, 35 mM AMP, 35 mM ATP or 35 mM adenosine along with 105 mM inorganic phosphate. Animals were analyzed 1½-2 hours after injections as described in the text. The original [^{3}H, α-^{32}p]ATP solutions had a ^{32}P/^{3}H ratio of 1.27. Data represent the average of two experiments. Pi stands for the inorganic phosphate, sodium phosphate.
+Total pool size.
Specific radioactivity.

Having thus described my invention, what I claim as new and desire to secure by Letters Patent is:

1. A method for increasing total liver ATP pools, total blood ATP pools and blood plasma ATP levels by administering to a mammalian host a member selected from the group consisting of: (a) a mixture of inorganic phosphate and adenosine; and, (b) an adenine nucleotide wherein said adenine nucleotide contains adenosine moiety(ies) and phosphate moiety(ies) and undergo rapid degradation to adenosine and inorganic phosphate after administration to said host.

2. The method of claim 1 wherein a mixture of inorganic phosphate and adenosine is administered to said host and wherein said inorganic phosphate is selected from the group consisting of sodium phosphate, potassium phosphate and phosphoric acid.

3. The method of claim 1 wherein said phosphate employed is an aqueous solution having a pH of about 6.0 to about 7.5.

4. The method of claim 1 wherein said phosphate employed is an aqueous solution having a pH of about 6.2 to about 7.0.

5. The method of claim 1 wherein the amount of combined adenosine and phosphate is about 1-1000 mg/kg of body weight per 24 hours and said administering is oral or topical.

6. The method of claim 1 wherein the amount of combined adenosine and phosphate is about 0.01-500 mg/kg of body weight per 24 hours and said administering is by injection.

7. The method of claim 1 wherein the amount of combined adenosine and phosphate is 0.001-10 mg/kg of body weight per minute and delivery is accomplished by infusion at this rate.

8. The method of claim 1 wherein said host is a human host.

9. The method of claim 1 wherein the source of adenosine and inorganic phosphate are adenine nucleotides that contain adenosine moiety(s) and phosphate moiety(s) and which undergo rapid degradation to adenosine and inorganic phosphate after administration to a host.

10. The method of claim 1 wherein at least about 1 equivalent of phosphate per equivalent of adenosine is employed.

11. The method of claim 1 wherein the molar ratio of adenosine to inorganic phosphate is about 1:1 to about 1:3.

12. A method for increasing the intracellular levels of ATP in a mammalian host by administering to said mammalian host a member selected from the group consisting of: (a) a mixture of inorganic phosphate and adenosine; and, (b) an adenine nucleotides wherein said adenine nucleotides contain adenosine moiety(ies) and phosphate moiety(ies) and undergo rapid degradation to adenosine and inorganic phosphate after administration to said host.

13. The method of claim 12 wherein at least about 1 equivalent of phosphate per equivalent of adenosine is employed.

14. The method of claim 12 wherein total liver ATP pool are increased by administering.

15. The method of claim 12 wherein said host is a human host.

16. The method of claim 12 wherein ATP levels of an organ is increased by said administering.

17. The method of claim 12 wherein the molar ratio of adenosine to inorganic phosphate is about 1:1 to about 1:3.

18. The method of claim 12 wherein adenosine 5'-monophosphate or adenosine 5'-triphosphate is administered to said host.

19. The method of claim 12 wherein an adenine nucleotide is administered to said host and wherein said adenine nucleotide contains adenosine moiety(ies) and phosphate moiety(ies) and undergo rapid degradation to adenosine and inorganic phosphate after administration to a host.

20. The method of claim 12 wherein the amount of combined adenosine and phosphate is about 1-1000 mg/kg of body weight per 24 hours and said administering is oral or topical.

21. The method of claim 12 wherein the amount of combined adenosine and phosphate is about 0.01-500 mg/kg of body weight per 24 hours and said administering is by injection.

22. The method of claim 12 wherein the amount of combined adenosine and phosphate is 0.001–10 mg/kg of body weight per minute and delivery is accomplished by infusion at this rate.

23. A method for increasing cellular level of ATP in an organ but not in red blood cells nor in blood plasma in a mammalian host by administering to said mammalian host adenosine.

24. The method of claim 23 wherein total liver ATP pools are increased by said administering.

25. The method of claim 23 wherein said host is a human host.

26. The method of claim 23 wherein ATP level of an organ is increased by said administering.

27. The method of claim 12 wherein a mixture of inorganic phosphate and adenosine is administered to said host and wherein said inorganic phosphate is selected from the group of sodium phosphate, potassium phosphate and phosphoric acid.

28. The method of claim 12 wherein said phosphate employed is an aqueous solution having a pH of about 6.0 to about 7.5.

29. The method of claim 12 wherein said phosphate employed is an aqueous solution having a pH of about 6.2 to about 7.0.

30. The method of claim 18 wherein adenosine 5'-monophosphate is administered to said host.

31. The method of claim 18 wherein adenosine 5'-triphosphate is administered to said host.

32. A method for increasing extracellular blood plasma levels of ATP in a mammalian host by administering to said host a member selected from the group consisting of: (a) a mixture of adenosine and inorganic phosphate; and, (b) an adenine nucleotides wherein said adenine nucleotides contain adenosine moiety(ies) and phosphate moiety(ies) and undergo rapid degradation to adenosine and inorganic phosphate after administration to said host.

33. The method of claim 32 wherein adenosine 5'-monophosphate is administered to said host.

34. The method of claim 32 wherein adenosine 5'-triphosphate is administered to said host.

35. The method of claim 32 wherein an adenine nucleotide is administered to said host and wherein said adenine nucleotides contain adenosine moiety(ies) and phosphate moiety(ies) and undergo rapid degradation to adenosine and inorganic phosphate after administration to said host.

36. The method of claim 18 wherein the amount of adenosine 5'-monophosphate or adenosine 5'-triphosphate is about 1–1000 mg/kg of body weight per 24 hours and said administering is oral or topical.

37. The method of claim 18 wherein the amount of adenosine 5'-monophosphate or adenosine 5'-triphosphate is about 0.01–500 mg/kg of body weight per 24 hours and said administering is by injection.

38. The method of claim 18 wherein the amount of adenosine 5'-monophosphate or adenosine 5'-triphosphate is 0.001–10 mg/kg of body weight per minute and delivery is accomplished by infusion at this rate.

39. The method of claim 19 wherein the amount of adenine nucleotide is about 1–1000 mg/kg of body weight per 24 hours and said administering is oral or topical.

40. The method of claim 19 wherein the amount of adenine nucleotide is about 0.01–500 mg/kg of body weight per 24 hours and said administering is by injection.

41. The method of claim 19 wherein the amount of adenine nucleotide is 0.001–10 mg/kg of body weight per minute and delivery is accomplished by infusion at this rate.

42. The method of claim 23 wherein the amount of adenosine is about 1–1000 mg/kg of body weight per 24 hours and said administering is oral or topical.

43. The method of claim 23 wherein the amount of adenosine is about 0.01–500 mg/kg of body weight per 24 hours and said administering is by injection.

44. The method of claim 23 wherein the amount of adenosine is 0.001–10 mg/kg of body weight per minute and delivery is accomplished by infusion at this rate.

45. The method of claim 1 wherein said member is in the form of a pharmaceutically acceptable salt.

* * * * *

The opinion in support of the decision being entered today was not written for publication and is not precedent of the Board.

Paper No. 21

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte ELIEZER RAPAPORT

Appeal No. 1996-3557
Application 08/131,948[1]

ON BRIEF

Before WILLIAM F. SMITH, SPIEGEL, MILLS, Administrative Patent Judges.

MILLS, Administrative Patent Judge.

DECISION ON APPEAL

This is a decision on appeal under 35 U.S.C. § 134 from the examiner's final rejection of claims 1-24, which are all of the claims pending in this application.

We reverse. A new rejection of the claims under 37 CFR § 1.196(b) is made.

[1] The filing date of this application is October 8, 1993.

Claim 1 is illustrative of the claims on appeal and reads as follows:

1. A method for treating Human Immunodeficiency Virus infection, and/or Human Immunodeficiency Virus disease, and/or Acquired Immunodeficiency Syndrome related complex, and/or Acquired Immunodeficiency Syndrome, and/or Acquired Immunodeficiency Syndrome with secondary infections, by administering to a human host a member selected from the group consisting of: (a) a mixture of adenosine and inorganic phosphate; and/or (b) an adenine nucleotide wherein said adenine nucleotide containing adenosine moiety(ies) and phosphate moiety(ies) wherein said adenine nucleotide undergoes rapid degradation to adenosine and inorganic phosphate after administration to said host.

The prior art references of record relied upon by the examiner in rejecting the appealed claims are:

Rapaport (U.S.)	5,049,372	Sep. 17, 1991

Lahdevirta et al. (Lahdevirta), "Elevated Levels of Circulating Cachectin/Tumor Necrosis Factor in Patients with Acquired Immunodeficiency Syndrome," The American Journal of Medicine, Vol. 85, pp. 289-91(1988).

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[2] Originally made of record as an attachment to Paper No. 3, filed May 2, 1994.

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[9] Originally made of record as an attachment to both Paper Nos. 5 (filed November 14, 1994) and 9 (filed February 14, 1995).

Zangerle et al., "Weight loss in HIV-1 infection is associated with immune activation," AIDS, Vol. 7, No. 2, pp. 175-81 (1993).

Reference cited by the merits panel:

Hofmann et al. (Hofmann AIDS), "Restoration of T-Cell function in HIV infection by reduction of intracellular cAMP levels with adenosine analogues," AIDS, pp.659-64 (1993).[4, 5]

OPINION

In reaching our decision in this appeal, we have given careful consideration to the appellant's specification and claims, to the applied prior art references, and to the respective positions articulated by the appellant and the examiner.

Rather than reiterate the conflicting viewpoints advanced by the examiner and the appellant regarding the above-noted rejection, we make reference to the Examiner's Answer (Paper No. 11, mailed June 7, 1995); Supplemental Examiner's Answer (Paper No. 14, mailed October 18, 1995); Supplemental Examiner's Answer (Paper No. 16, mailed January 17, 1996); and Supplemental Examiner's Answer (Paper No. 18, mailed April 1, 1996) for the examiner's complete reasoning in support of the rejection, and to the appellant's Brief (Paper Nos. 9 and 10, filed February 14, 1995); Reply Brief (Paper No. 12, filed August 2, 1996); Second Reply Brief (Paper No. 15, filed December 21, 1995) and Third Reply Brief (Paper No. 17, filed February 13, 1996) for the appellant's

[4] According to the Patent and Trademark Office Scientific and Technical Information Center, Hofmann AIDS published May 1, 1993 (see attached).

[5] Hofmann AIDS was cited in the specification at page 14, lines 15-28, in Proposal, Drugs, and in the Reply Brief page 9, reference 23.

arguments thereagainst. As a consequence of our review, we make the determinations which follow.

Issues

1. Claims 1-24 stand rejected under the doctrine of obviousness-type double patenting over claims 1-45 of Rapaport in view of Lahdevirta and Peters.

2. Claims 1-24 stand rejected under 35 U.S.C. § 103 over Rapaport in view of Lahdevirta and Peters.

3. Claims 1-24 stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement.

Obviousness-type double patenting and 35 U.S.C. § 103

Claims 1-24 stand rejected under the doctrine of obviousness-type double patenting over claims 1-45 of Rapaport in view of Lahdevirta and Peters. Claims 1-24 stand rejected under 35 U.S.C. § 103 over Rapaport in view of Lahdevirta and Peters.

It is the examiner's position that Rapaport "teaches that the administration of adenosine or adenine nucleotides (AMP, ADP, ATP) prevents cachexia in animals having cancer." Examiner's Answer, page 6. Rapaport does not teach the administration of such compounds for the treatment of AIDS.

Lahdevirta is relied on for the disclosure that cachexia is typically a component of AIDS. Peters is relied on for the disclosure that many AIDS patients have cancer, such as Kaposi's sarcoma and lymphoma. Thus, the examiner argues that,

> Since adenosine and adenine nucleotides can be used to treat cachexia and cancer, and cachexia and cancer are symptoms of AIDS, it would have been obvious that administration of adenosine or adenine nucleotides would be a beneficial treatment for AIDS patients suffering from cancer and/or cachexia.

Examiner's Answer, page 7.

Assuming arguendo that the examiner has established a prima facie case of obviousness, the burden then falls on an appellant to rebut that prima facie case. Such rebuttal or argument can consist of any other argument or presentation of evidence that is pertinent. In re Dillon, 919 F.2d 688, 692-93, 16 USPQ2d 1897, 1901 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991). Accordingly, we carefully evaluate the objective evidence of nonobviousness supplied by the appellant.

The appellant argues and presents evidence that HIV wasting syndrome is widely acknowledged not to be mechanistically understood and that HIV wasting syndrome (AIDS-related cachexia) is different in certain aspects from cancer cachexia. Reply Brief, page 3. Moreover, appellant argues and provides evidence that weight loss in AIDS is directly related to events associated with immune activation which is unique to advanced AIDS and is not a clinical factor in advanced cancers. Reply Brief, page 4.

In response to appellant's argument, the examiner suggests that many AIDS patients suffer from cancer and that appellant has not made it clear why one of ordinary skill in the art would not use the treatment of Rapaport to treat cancer in AIDS patients, given the high rate of cancer in AIDS patients. What is missing from the examiner's

rebuttal position is evidence or facts which address appellant's specific evidence and argument that AIDS cachexia is different than the cancer cachexia treated by Rapaport. Rapaport, column 3, lines 63-68. While it would seem reasonably clear that cancer can be treated by the method of Rapaport as evidenced by the patent, the direct correlation of Rapaport to the treatment of AIDS appears to be questioned by the evidence presented by appellant as to the differences between AIDS cachexia and cancer cachexia. Conclusions of obviousness must be based upon facts not generalities. See In re Warner, 379 F.2d 1011, 1017, 154 USPQ 173, 178 (CCPA 1967), cert. denied, 389 U.S. 1057 (1968); In re Freed, 425 F.2d 785, 165 USPQ 570, 571 (CCPA 1970).

After evidence or arguments are submitted by the appellant in response to a rejection based on obviousness, patentability is determined on the totality of the record, by a preponderance of evidence with due consideration to persuasiveness of the argument. We have carefully studied the arguments and evidence of record. On balance, we believe that the totality of the evidence presented by the examiner and appellant weighs in favor of non-obviousness of the claimed invention. The rejection of the claims for obviousness of the claimed invention is reversed.

New grounds of rejection - 37 CFR § 1.196(b)

Under the provisions of 37 CFR § 1.196(b), we enter the following new grounds of rejection against appellant's claims 1-24.

Claims 1-24 are rejected under the judicially created doctrine of obviousness type double patenting in view of Rapaport and Hofmann AIDS.

Claims 1-24 are rejected under 35 U.S.C. § 103 as being unpatentable in view of Rapaport and Hofmann AIDS.

Claim 1

The claimed invention is directed to

1. A method for treating Human Immunodeficiency Virus infection, and/or Human Immunodeficiency Virus disease, and/or Acquired Immunodeficiency Syndrome related complex, and/or Acquired Immunodeficiency Syndrome, and/or Acquired Immunodeficiency Syndrome with secondary infections, by administering to a human host a member selected from the group consisting of: (a) a mixture of adenosine and inorganic phosphate; and/or (b) an adenine nucleotide wherein said adenine nucleotide containing adenosine moiety(ies) and phosphate moiety(ies) whereas said adenine nucleotide undergoes rapid degradation to adenosine and inorganic phosphate after administration to said host.

Rapaport claims and discloses a process for selectively arresting the growth of tumor cells in a host which comprises administering to a host having tumor cells adenosine 5'-monophosphate, or pharmaceutically acceptable salts thereof, or chelates thereof or liposomes thereof, or radio-nuclides thereof in an amount sufficient to increase the blood and plasma levels of adenosine 5'-triphosphate in said host sufficiently to thereby arrest the growth of said tumor cells (Claim 1) However, Rapaport also claims and discloses a more general process for increasing the blood and plasma levels of adenosine 5'-triphosphate in a host which comprises administering at least one compound selected from the group of adenosine 5'-monophosphate, adenosine 5'-diphosphate, adenosine 5'-triphosphate, pharmaceutically acceptable

salts thereof, chelates thereof liposomes thereof, and radio-nuclides thereof to said host in an amount sufficient to increase said blood and plasma levels (Claim 29).

The difference between the claimed invention and the process of Rapaport is that Rapaport does not provide an indication that the adenine nucleotides administered in the process of Rapaport may be used for the treatment of AIDS and/or HIV.

Hofmann AIDS discloses the restoration of T-cell function in HIV infection by reduction of intracellular cAMP levels with the adenosine analogues, such as 2',5'-dideoxyadenosine (ddAdo), or with 3'AMP (adenosine monophosphate). Hofmann AIDS, page 662, column 2, and page 663, column 1. Hofmann AIDS discovered that high intracellular cAMP concentrations contribute to the pathogenesis of T-cell anergy in HIV infection and that drugs, such as ddAdo and 3'AMP, which decrease intracellular cAMP levels may be beneficial in the treatment of AIDS (see abstract). Data in Hofmann AIDS show that cytotoxic T-cells from HIV seropositive subjects regained their normal ability to lyse an allogenic tumor cell line in the presence of adenylate cylclase inhibitor, ddAdo, in six out of six experiments. Hofmann AIDS, page 661.

Hofmann AIDS found that a reduction of abnormal cAMP levels restored proliferative and cytotoxic function of T-cells, and determined that the adenosine analogue, ddAdo, or 3'AMP inhibit adenylate cyclase, the enzyme responsible for the generation of cAMP, by competition for the 'P' binding site. Hofmann AIDS, page 663,

column 2. Hofmann AIDS suggests that restoration of T-cell functions should be of great benefit in the treatment of HIV infection and suggests that clinical benefit could be derived from improved immunity to the opportunistic infections common in AIDS. It is also posited that the improved function in vivo may be possible by treatment with the agents described in vitro in Hofmann AIDS. Hofmann AIDS, page 663, column 2.

It would have been obvious to one of ordinary skill in the art at the time of the present invention to use the process of Rapaport for increasing blood and plasma levels of ATP by administering adenosine monophosphate, for the treatment of HIV and AIDS, in view of the disclosure of Hofmann AIDs of the restoration of T-cell function in HIV infection by reduction of intracellular cAMP levels with the adenosine analogue ddAdo and 3'AMP. The above analysis of the independent claims and the stated motivation or suggestion for the combination of the cited references also supports the rejection of the independent and dependent claims indicated below.

Claims 2 and 18

Claims 2 and 18 require that the adenine nucleotides are administered to the host in the form of pharmaceutically acceptable salts thereof, or chelates thereof, or metal complexes thereof, and is described in the Rapaport abstract.

Claims 3-8 and 22-24

Claims 3-8 and 22-24 require specific amounts and administration routes for adenosine and adenine nucleotide which are described in Rapaport, generally, in claims 1-45, particularly claims 2-5, 15 and 30-35.

Claims 9-12

Claims 9-12 requires the administration of adenosine and inorganic phosphate, AMP or ATP and is described in Rapaport, claims 10-12 and 19-21.

Claim 13

Claim 13 requires a pH of 6.0 to 7.5 and is described in Rapaport at column 12, line 13 and column 13, line 13.

Enablement

Claims 1-24 stand rejected under 35 U.S.C. § 112, first paragraph for lack of enablement.

In order to sustain a rejection under 35 U.S.C. § 112, first paragraph, for lack of enablement, the examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention. See In re Wright, 999 F.2d 1557, 1561-62, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993).

The examiner sets forth a prima facie case of lack of enablement by fairly considering the factors set forth in In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404, (Fed. Cir. 1988):

> Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman, [230 USPQ 546, 547 (Bd. Pat. App. Int. 1986)]. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims. (footnote omitted).

See Examiner's Answer, pages 3-6.

However, it is also well settled that to satisfy the enablement requirement, an application need not teach, and preferably omits, that which is well known in the art. How such a teaching is set forth, whether by the use of illustrative examples or by broad descriptive terminology, is of no importance since the specification which teaches how to make and use the invention in terms which correspond in scope to the claims must be taken as complying with the first paragraph of 35 U.S.C. § 112 unless there is reason to doubt the objective truth of the statements relied upon therein for enabling support. See In re Marzocchi, 439 F.2d 220, 223-24, 169 USPQ 367, 369-70 (CCPA 1971).

Appellant responds to the examiner's prima facie case of lack of enablement with argument that 1) there is no animal or in vitro experimental model for the spectrum of physiological functions adversely affected by HIV disease/AIDS addressed by appellant's invention, and 2) that the clinical proposal for the utilization of ATP in advanced AIDS documents the expected efficacy of the present invention, referencing the experimental data of 61 references (proposal, page 18) and 79 references (proposal, page 35). Appeal Brief, page 10. Appellant references the Hofmann AIDS publication in the clinical proposal, and the data therein, as supporting enablement of the present application. Appeal Brief, page 10 and Reply Brief, pages 9-12. Appellant suggests that references cited in the clinical proposal are correlated with specific effects

of elevated ATP or adenosine, and support enablement.[6] Appellant argues that this evidence of the state of the art, when read with the specification, would enable the practice of the invention by one of ordinary skill in the art with a reasonable expectation of success.

It would appear that the examiner has been hasty to discount the evidence of the state of the art provided by appellant in support of enablement, such as Hofmann AIDS referenced in the clinical proposal. The examiner urges that the clinical proposal or plan presented by appellant is mere hypothesis, does not present experimental data and is not a "result".[7] This general treatment of the proposal by the examiner is not sufficient rebuttal to the evidence presented in Hofmann AIDS and other references cited therein in support of enablement. Having failed to rebut appellant's evidence submitted regarding enablement, the rejection of the examiner of the claims under 35 U.S.C. § 112, first paragraph is reversed.

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[6] See Paper No. 3, page 7; Paper No. 5, page 8 and Reply Brief, page 9.

[7] The examiner also urges that none of the references cited in the clinical or research proposal have been made of record. Supplemental Examiner's Answer, page 7. This however, does not mean their reference in the proposal of record can be ignored. In addition, the Proposal was originally submitted as an attachment to Paper No. 3, filed May 2, 1994, which is very similar to the content of Drugs, made of record as an attachment to Paper No. 5. Hofmann AIDS was cited in the specification at page 14, lines 15-28 and in the Reply Brief page 9, reference 23.

CONCLUSION

The decision of the examiner to reject Claims 1-24 for obviousness type double patenting and under 35 U.S.C. § 103 is reversed. However, claims 1-24 are rejected pursuant to the provisions of 37 CFR. § 1.196(b) as being obvious in view of Rapaport and Hofmann AIDS. The rejection of the examiner of the claims under 35 U.S.C. § 112, first paragraph is reversed.

TIME PERIOD FOR RESPONSE

This decision contains a new ground of rejection pursuant to 37 CFR § 1.196(b). 37 CFR § 1.196(b) provides that, "[a] new ground of rejection shall not be considered final for purposes of judicial review."

37 CFR § 1.196(b) also provides that the appellant, WITHIN TWO MONTHS FROM THE DATE OF THE DECISION, must exercise one of the following two options with respect to the new ground of rejection to avoid termination of proceedings (§ 1.197(c)) as to the rejected claims:

> (1) Submit an appropriate amendment of the claims so rejected or a showing of facts relating to the claims so rejected, or both, and have the matter reconsidered by the examiner, in which event the application will be remanded to the examiner. . . .

(2) Request that the application be reheard under § 1.197(b) by the Board of Patent Appeals and Interferences upon the same record. . . .

No time period for taking any subsequent action in connection with this appeal may be extended under 37 CFR § 1.136(a).

<u>REVERSED. 1.196(b)</u>.

William F. Smith
WILLIAM F. SMITH
Administrative Patent Judge

Carol A. Spiegel
CAROL A. SPIEGEL
Administrative Patent Judge

Demetra J. Mills
DEMETRA J. MILLS
Administrative Patent Judge

) BOARD OF PATENT
) APPEALS AND
) INTERFERENCES